Activity 3

Cancer as a Multistep Process

Focus: Students use random number tables and a CD-ROM-based simulation to test several hypotheses about the development of cancer.

Major Concepts: No single event is enough to turn a cell into a cancerous cell. Instead, it seems that the accumulation of damage to a number of genes ("mul tiple hits") across time leads to cancer.

Objectives: After completing this activity, students will

- understand that cancer results from the accumulation of genetic damage to cells across time and
- be able to explain the increase in cancer incidence that occurs with an increase in age in terms of a multiple hit (mutations in a number of genes) hypothesis for cancer's development.

Prerequisite Knowledge: Students should be familiar with the concepts taught in Activities 1 and 2. Students also should have a basic knowledge of probabil ity. The annotation to Step 6 describes a short exercise you can do with students to remind them of the laws of probability.

Basic Science-Public Health Connection: This activity highlights the contribu tion epidemiology has made to our understanding of cancer. Students discover how determining and analyzing the frequencies with which cancer occurs in large populations has provided compelling though indirect evidence that human cancer is a multistep process. Students then consider the implications of this understanding of cancer for personal and public health.

The process by which a normal cell is transformed into a malignant cell involves many changes. Cancer cells display a host of striking differences from their normal counterparts, such as shape changes, changes in their dependence on growth factors, and a multitude of biochemical differences.

One of the earliest questions scientists asked about these phenotypic differences was how they are generated. Another question was whether these differences arise all at once, at a moment when the cell experiences a sudden, catastrophic shift from "normal" to "malignant," or gradually, across time, as a result of many small events, each contributing yet another characteristic to a set that in sum gives the cell a malignant phenotype.

In this activity, students examine some of the epidemiologic data that suggest that the development of cancer is a multistep process. Students study a graph of colon cancer incidence by age, answer an initial set of questions about the relative risk of developing colon cancer at various ages, and propose answers to

the question of why this risk increases with age. Students then use random number tables and a CD-ROM-based simulation to test several hypotheses about the development of colon cancer (for example, colon cancer develops as a result of a single event within a cell, colon cancer develops as a result of two independent events within a cell, and so on). Finally, students use their understanding of the development of cancer as a multistep process to explain (1) the increased incidence of cancer with age, (2) the development of cancer decades after exposure to known carcinogens, and (3) the increased incidence of cancer among people with inherited predispositions.

Materials and Preparation

You will need to prepare the following materials before conducting this activity:

- Master 3.1, *Colon Cancer Incidence by Age* (make 1 copy per student and 1 transparency)
- Master 3.2, *Random Number Tables* (make enough copies to have 1 data set per student)

   Copy the master and cut out one data set for each student in your class.

- Master 3.3, *Collecting the Data* (make 1 transparency)
- Master 3.4, *Graphing the Data* (make 1 copy per student and 1 transparency)
- Master 3.5, *Using the Hit Simulator* (make 1 copy per student)
- Master 3.6, *Testing an Explanation by Looking at Additional Data* (make 1 copy per student)
- coins (1 penny, 1 nickel, and 1 dime for each student, only if you plan to conduct the review of probability described in Step 6)
- a hat (or other container) with folded slips of paper containing the numbers from 1 to 25
- *Cell Biology and Cancer* CD-ROM

Follow the instructions on pages 28–29 to load the CD-ROMs on the computers the students will use.

Note to teachers: If you do not have enough computers equipped with CD-ROM drives to conduct this activity, you can use the print-based alternative. To view and print the instructions and masters for this alternate activity, load the CD onto a computer and click the Print button on the main menu screen. The computer will display a screen showing the resources available for printing from the CD; click on the button labeled Non-CD Lesson Plan from the choices available for Activity 3, *Cancer as a Multistep Process.* This will reveal the lesson plan and the masters for the alternate, non-CD-based lesson. Click Print again to print these resources.

Procedure

1. Open the activity by reminding students of the increase in cancer incidence with age that they observed in Activity 1. Explain that in this activity, they will investigate the biological basis for this increase.

It is very important to set this activity in the context of Activities 1 and 2.

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Without this context, students may complete this activity "by rote" and never see how it relates to our growing understanding of the biological basis of cancer.

2. **Distribute one copy of Master 3.1, *Colon Cancer Incidence by Age*, to each student and ask the students to work in pairs to answer the questions below the graph.**

Give students about 5 minutes to complete this task.

3. **Display a transparency made from *Colon Cancer Incidence* and invite the students to share their answers to the questions.**

   Question 1 How likely is it that you will develop colon cancer this year?

   Students should answer that the risk is so low that they cannot read it from the graph. You may wish to ask whether children under 15 ever develop colon cancer. In fact, those few children who do have genetic conditions that predispose them to the development of cancer.

   Question 2 How likely is it that someone who is 60 years old will develop colon cancer this year?

   The risk is significantly higher (approximately 70 per 100,000 persons).

   Question 3 How likely is it that someone who is 80 years old will develop colon cancer this year?

   This risk is even higher (about 350 per 100,000 persons).

   Question 4 How can we explain this change in the risk of a person developing colon cancer?

   Answers will vary. Students may suggest that as people age, they become more susceptible to cancer. Some may also suggest that it takes time for the mutations involved in the development of cancer to accumulate. Accept all reasonable answers, explaining that in this activity, students will have a chance to test a possible answer to this question.

4. **Circle the last question on the transparency or write it on the board and point out that this question is the central issue in this activity. Explain that many years ago epidemiologists recognized that this change in cancer risk provided an important clue about the cause of cancer. This activity challenges students to retrace the thinking of these scientists and discover this clue for themselves.**

   If students are unfamiliar with the term "epidemiology," explain that it is the study of the incidence of disease in a population.

5. **Remind students that one way scientists answer questions is by developing and testing hypotheses, or tentative explanations. For example, one explanation that might be offered for the development of cancer might be summarized as "One mutation in a certain gene in a cell causes that cell to become**

cancerous" (the one-hit hypothesis). Another explanation might be summa-rized as "Two mutations in separate genes of a cell are required before the cell becomes cancerous" (the two-hit hypothesis), and so on. Ask students if they can tell by looking at the colon cancer graph which of these two expla-nations for the development of cancer best explains the data.

Students likely will answer that they cannot tell just by looking at the graph.

6. Explain further that scientists often use models to test their explanations. In this activity, students will use two simple models, one involving ran-dom number tables and the other using a simulation on the CD-ROM, to test several alternate explanations for the development of cancer.

If your students are not familiar with some of the basic concepts of probability, you may wish to conduct the following short exercise:

Give each student a penny, a nickel, and a dime, and ask students to toss each coin one time and leave the coins lying on their desks where they landed. Ask the students to raise their hands if they got a "heads" on their penny. Count the number of students who raise their hands and point out that this represents about 50 percent of the class. Then, ask stu dents to indicate how many got heads on both their penny and their nickel. Again, count the number of students who raise their hands and point out that this value is close to 25 percent of the class. Finally, ask stu dents to raise their hands only if they got a heads on all three of their coins (the penny, the nickel, and the dime). This number should be about one-eighth of the class. Ask students what pattern they see in these data. Students should see that the probability of independent events happen ing together is lower than each event's individual probability. Use your judgment to decide whether to explain to students how to calculate the probability of such occurrences (for example, the probability of getting heads on three coins tossed individually is $1/2 \times 1/2 \times 1/2 = 1/8$).

7. Distribute one data set from Master 3.2, *Random Number Tables*, to each student and explain that students will use these data to understand the implications of the following two hypotheses for the incidence of cancer in a population (the class):

1. Cancer develops as a result of a single mutation (one-hit hypothesis).
2. Cancer develops as a result of two independent mutations (two-hit hypothesis).

8. Explain that the data sets the students hold are called random number tables and were made as a computer randomly chose numbers between 1 and 25 to correspond with the students' imagined life spans. Explain that the first column on the table represents the students' ages, and that the second and third columns on the table represent the numbers the com-puter chose.

9. **Conduct the following exercise:**

   • **Ask a student to draw a number out of the hat and announce the number to the class. Write the number on the board.**

   For example, imagine that the student drew the number 10.

   • **Explain that this number represents a mutation in gene 1. Ask students to examine the column labeled "Gene 1" on their random number table to determine whether they have the number chosen. If they do, they should circle it and note the age at which it occurred.**

   Students should look down the column labeled "Gene 1" for the first occurrence of the "unlucky" number (in this example, 10). If the number occurs more than once, they should ignore the second (and any subsequent) occurrence.

   • **Ask another student to draw a number out of the hat and announce it to the class. Write the number on the board.**

   For example, imagine that the student drew the number 4.

   • **Explain that this second number represents a mutation in gene 2. Ask students to examine the column labeled "Gene 2" on their random number table to determine whether they have the second number chosen. If they do, they should circle it and note the age at which it occurred.**

   Students should look down the column labeled "Gene 2" for the first occurrence of the "unlucky" number (in this example, 4). If the num ber occurs more than once, they should ignore the second (and any subsequent) occurrence.

10. **Display a transparency made from Master 3.3, *Collecting the Data*, and explain that you are going to use this table to tally the number of people in the class who would have developed cancer at each age if the one-hit or two-hit hypotheses for the development of cancer were true.**

    Explain that to discover the number of people who would have developed cancer, the students need to examine their random number tables according to the following instructions:

    • **Tell students that first the class is going to approximate what might happen if the one-hit hypothesis were true (that is, if one mutation were sufficient to cause a normal cell to become cancerous). Ask students to imagine that if they found the first "unlucky" number in the column labeled "Gene 1," it meant a gene in one of their cells experienced a cancer-causing mutation. Explain that if the one-hit hypothesis were correct, the age at which the unlucky number *first* appears in the column labeled "Gene 1" would be the age at which they developed colon cancer.**

Note that some students likely will not encounter the unlucky number and, therefore, will not develop cancer.

- **Poll the class to determine how many students developed cancer under this model at each age (5–100 years) and fill this information into the column labeled "Number of People Who Developed Cancer—One Hit" on the transparency.**

Only students who had the first unlucky number (in the example, 10) in the column labeled "Gene 1" should indicate that they developed cancer.

- **Next, tell students that the class is going to approximate what might happen if the two-hit hypothesis were true (that is, if two mutations were required to cause a normal cell to become cancerous). Ask students to imagine that if they encountered the second "unlucky" number in the column labeled "Gene 2," it meant they experienced a cancer-causing mutation in another gene in the same cell that experienced the first mutation. Explain that the age at which the second unlucky number first appears in the column "Gene 2" is the age at which they experienced this second mutation. However, if the two-hit hypothesis were correct, they did not develop colon cancer until the age at which they encountered *both* unlucky numbers (the unlucky number in the column labeled "Gene 1" *and* the unlucky number in the column labeled "Gene 2").**

Tell students that it does not matter in what order the mutations occur. Emphasize that in order to have developed cancer under this model, they would need to have both the first unlucky number in the column labeled "Gene 1" *and* the second unlucky number in the column labeled "Gene 2" (in the example, both a 10 in the first column and a 4 in the second column). The age at which they developed cancer is the age by which they had experienced *both* mutations.

Note that many students likely will not encounter both unlucky numbers and, therefore, will not develop cancer.

- **Poll the class to determine how many students developed cancer at each age under this second model and display the data in the appropriate column on the transparency.**

- **Work with the class to accumulate the running total of cancer cases observed in the class population at each age under the one-hit and two-hit models and write these values into the last two columns on the transparency.**

These running totals represent the cumulative number of people who developed colon cancer at or before each age.

11. **Ask students whether they see any pattern in the incidence of cancer in this population (the class).**

Students may see that fewer people developed cancer under the second model (the two-hit hypothesis) than under the first (the one-hit hypothesis) and that those who did develop cancer under the two-hit hypothesis tended to do so later in life. Encourage students to express this observation as a generalization (for example, the chance, or "risk,"of developing cancer early in life diminished as the number of events involved in its development increased). If students are able to recognize this, ask them what this observation suggests about the development of cancer. *Do not give students this answer—that it suggests that more than one event may be involved in the development of cancer—but indicate that they should think about this question as they complete the activity.*

12. **Distribute one copy of Master 3.4, *Graphing the Data*, to each student and direct students to work in pairs to construct two graphs that illustrate the chances that a person in this class would have developed colon cancer by a certain age if the one-hit or two-hit hypothesis were true.**

13. **Ask students to examine the graphs they generated in Step 12 and decide which hypothesis best fits the actual data on the incidence of colon cancer.**

    Students likely already recognize that fewer cancer cases were encountered when two mutations (two hits) were required. However, they also may see from the graph that neither hypothesis fits the colon cancer data well.

14. **Ask students to predict what the results of a simulation such as this might be if three, four, or as many as five mutations (five hits) must occur prior to the onset of cancer.**

    Students may be able to suggest that cancer would become an increasingly rare event and would tend to occur later and later in life as the number of mutations required for its development increases.

15. **Distribute one copy of Master 3.5, *Using the Hit Simulator*, to each student. Direct students to organize into their teams and follow the instructions provided to use the CD-ROM-based simulation to test their predictions and decide what type of model for the development of colon cancer best fits its observed incidence.**

    This simulation is a sophisticated tool that students can use to observe how mutation frequency and the number of mutations (hits) required for the development of cancer affect the incidence of cancer in a population. Although students can simply experiment with the simulation, their experience likely will be more meaningful if they follow the guidelines provided on *Using the Hit Simulator*.

    Give students approximately 20 minutes to learn to use the simulation, test their predictions, and answer the questions on *Using the Hit Simulator*.

These questions focus students' attention on the activity's major con cepts. Encourage stu dents to express their understanding of cancer using the language of cells and genes.

16. Convene a class discussion in which you invite students to share their answers to the questions on *Using the Hit Simulator.*

• Investigate the Effect of Changing the Number of Hits Required

Question 1 How does the incidence of cancer change as you require a greater number of hits for a cell to become cancerous?

Students should see that the greater the number of hits required, the fewer the number of people who develop cancer and the later in life they tend to develop it. Students may note that with the number of hits set at 1 and the mutation rate set at 0.5 (50 percent), nearly every-one in the population gets cancer by age 25. As they increase the number of hits required, the curve shifts to the right (people get can cer later in life), though most people still develop it eventually.

Question 2 Recall the graph of the incidence of colon cancer that you observed at the beginning of this activity. Did the incidence of cancer in any of the runs you just completed match the incidence of cancer recorded in that graph? Explain your answer.

Students should recognize that none of the runs matched the actual incidence of colon cancer. You may wish to remind students that they learned in Activity 1 that only about 1 in 3 people in the United States develops cancer sometime in his or her life.

Question 3 What can you conclude from this observation?

Students should see that because the curve shifted to the right (toward the development of cancer later in life) as more hits were required, the results suggest that more than 1 hit likely is involved in the development of cancer. Astute students also may say that because almost everyone eventually developed cancer in these simulations, the mutation rate of 0.5 (50 percent) likely is too high.

• Investigate the Effect of Changing the Mutation Rate

Question 4 How does the incidence of cancer change as the muta-tion rate increases?

The incidence of cancer increases as the mutation rate increases. Students should see that with the simulator set at a mutation rate of 0.1 (10 percent), a smaller proportion of the population develops can cer than when the simulator is set at 0.5 (50 percent). With the simu lator set at a mutation rate of 1 (100 percent), everyone gets cancer between the ages of 0 and 5.

Question 5 Recall the graph of the incidence of colon cancer that you observed at the beginning of this activity. Did the incidence of cancer in any of the runs you just completed match the incidence of cancer recorded in that graph? Explain your answer.

Students should recognize that none of the runs matched the actual incidence of colon cancer. They should recognize, however, that the curve(s) generated with the mutation rate set at 0.1 or lower was/were more in line with the observed incidence than the curves generated with higher mutation rates.

Question 6 What can you conclude from this observation?

Students should recognize that these results suggest that the actual mutation rate is somewhat lower than 0.5 (50 percent), and maybe even lower than 0.1 (10 percent).

- **Investigate the Effect of Changing Both the Number of Hits Required and the Mutation Rate**

  Question 7 What can you conclude from your observations?

  Students should see that the curves generated by some of these runs begin to resemble the incidence of colon cancer observed on the graph they examined at the beginning of the activity. Encourage students to suggest combinations of numbers of hits and mutation rates that seem to give realistic results, but caution students not to use this simulator (which was designed for educational purposes*, not research) to try to make an absolute determination of number of hits and mutation rate.

  You may wish to point out that a mutation rate of 0.04 is the same rate that was used in the random number table exercise. Challenge students to demonstrate this by comparing the graphs they made of the one-hit and two-hit hypotheses with the curves generated by the simulator. Note that using the CD-ROM-based simulator allows them now to test the predictions they made in Step 14.

  *Note that the graph of the incidence of colon cancer used in Step 2 is actually the number of people in a population of 100,000 who will be diagnosed with colon cancer at each age. The graphs stu dents created in class and the graphs generated by the *Hit Simulator* are somewhat different because they plot cumulative numbers (the total number of people who will have developed colon cancer at or before that age).
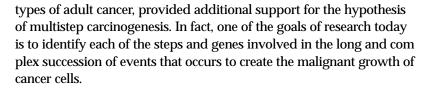
- **Summary**

  Question 8 What clue did the change in risk of colon cancer pro-vide scientists about the cause of cancer?

  Students should be able to explain that the increase with age in colon cancer incidence suggested to epidemiologists that more than one mutational event was involved in cancer's development. Similar graphs of age-dependent cancer incidence, plotted for many other

Whereas Activity 2 illus trates the contribution that cell biologists and geneticists have made to understanding cancer, Activity 3 illustrates the contribution that epi demiologists have made. One of the most exciting aspects of cancer research in recent years has been the construc tion of an understanding of cancer that unifies the work of many types of scientists studying can cer for more than 100 years.

types of adult cancer, provided additional support for the hypothesis of multistep carcinogenesis. In fact, one of the goals of research today is to identify each of the steps and genes involved in the long and com plex succession of events that occurs to create the malignant growth of cancer cells.

Note that this question returns students to the challenge they were given in Step 4.

17. Challenge your students to evaluate the models they used to test the different hypotheses for the development of cancer (that is, to think about the ways in which the random number table exercise and the CD-ROM-based simulation do and do not match reality).

Remind students that all models are inaccurate in some respects. For example, the mutational events within cells may not be completely ran dom, as the models assume. The models also assume that the probabil ity of each individual mutational event is the same, and this may not be the case. There is some evidence, for example, that some mutations increase the probability that other mutations will occur. In addition, the models do not consider that some mutations may be detected and repaired. Nevertheless, the fact that the models the students used are not perfect does not mean they are not useful tools for understanding how disease processes work.

18. Close the activity by distributing one copy of Master 3.6, *Testing an Explanation by Looking at Additional Data*. Ask students to use their understanding of cancer as a multistep process to explain each of the observations listed.

Question 1 Cancer is a disease of aging.

Students should be able to explain it takes time for all of the muta tions involved in the development of cancer to accumulate, and that this explains why the incidence of cancer increases with age (that is, why cancer is more likely to "strike" in the middle or later years than in childhood, youth, or young adulthood.

Question 2 You've come a long way, baby.

Students should be able to explain that as these women began to smoke, they began to accumulate cancer-causing mutations in their lung cells. Because the accumulation of these mutations to the point where a cell becomes cancerous takes time, the results of the increase in the number of women smoking (in the form of an increase in lung cancer among women) did not begin to appear for 20 to 25 years.

Question 3 Genes and increased susceptibility.

Students should recognize that if a person is born with a cancer-causing mutation already present in his or her cells, he or she has

The questions on *Testing an Explanation* are chal lenging, but they repre sent an excellent oppor tunity for you to evaluate your students' understanding of the activity's major concepts and their ability to apply their understanding to novel situations.

already experienced the first step toward the development of cancer and, thus, has a higher risk of accumulating all of the mutations required for the development of cancer than a person who does not carry such a mutation.